THE NUCLEOTIDE SEQUENCE OF TURTLE (*TERRAPENE CAROLINA*) 5 S RIBOSOMAL RIBONUCLEIC ACID

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1. Introduction

The sequence of bases in eukaryotic 5 S rRNA has been highly conserved, and this is particularly true within the mammals where all species examined have the same sequence as the human 5 S rRNA [1–5]. Amongst the lower vertebrates some differences in 5 S rRNA sequence have been reported [6–11], but in these cases only a single species of each class was studied. This paper reports the sequence of the 5 S rRNA of the turtle *Terrapene carolina,* and demonstrates that this sequence differs from that of iguana [11]. This is the first example of differences in the 5 S rRNA sequences of two members of the same vertebrate class.

2. Materials and methods

The cells used in this study were the TH-1 line of heart cells from *Terrapene carolina,* established by Clark et al. [12], and obtained from the ATCC (CCL 50). Labeling of RNA by growth of the cells in [^{32}P]orthophosphate-containing medium, isolation of the labeled 5 S rRNA, and its subsequent enzymic digestion were performed as described previously [11,13]. Standard procedures were used to separate the products of RNAase T_1 and pancreatic RNAase digestions [14]. The sequences of the separated oligonucleotides were also determined by well established methods, with specific examples described in the next section.

3. Results

Figure 1 shows the RNAase T_1 fingerprint of turtle 5 S rRNA. The products are identified in table 1, together with the methods used to determine their sequences. The pancreatic RNAase fingerprint is in fig.2, with the identification of spots in table 2. All oligonucleotides isolated, with the exception of t-18 and t-20, were completely sequenced to demonstrate the absence of sequence changes undetectable by electrophoretic mobility.

The products which differ from the corresponding mammalian and/or *Iguana* products are shown in fig.3.

Position 2 is identified as U as a result of isolation of the 5′-terminal pancreatic RNAase products (pp)pGpUp and also the modified U*C after carbodimide blocking of t-16, followed by pancreatic RNAase digestion. Position 3 is known to be C because of the isolation of U*AC from the same oligonucleotide, and the same reaction.

The A at position 25 was identified, as in the iguana study [11], by spleen phosphodiesterase digestion of t-9, and the absence of both AACG and a full molar equivalent of CG. This also accounts for the presence of an additional AC and the loss of one GC in the pancreatic RNAase digests, when compared to mammalian digests.

The absence of the tetranucleotide ACCG and one molar equivalent of CCUG, when compared to mammalian and iguana digests, is accounted for by t-18, the sequence of which is not completely determined due to poor yields of spleen phosphodiesterase

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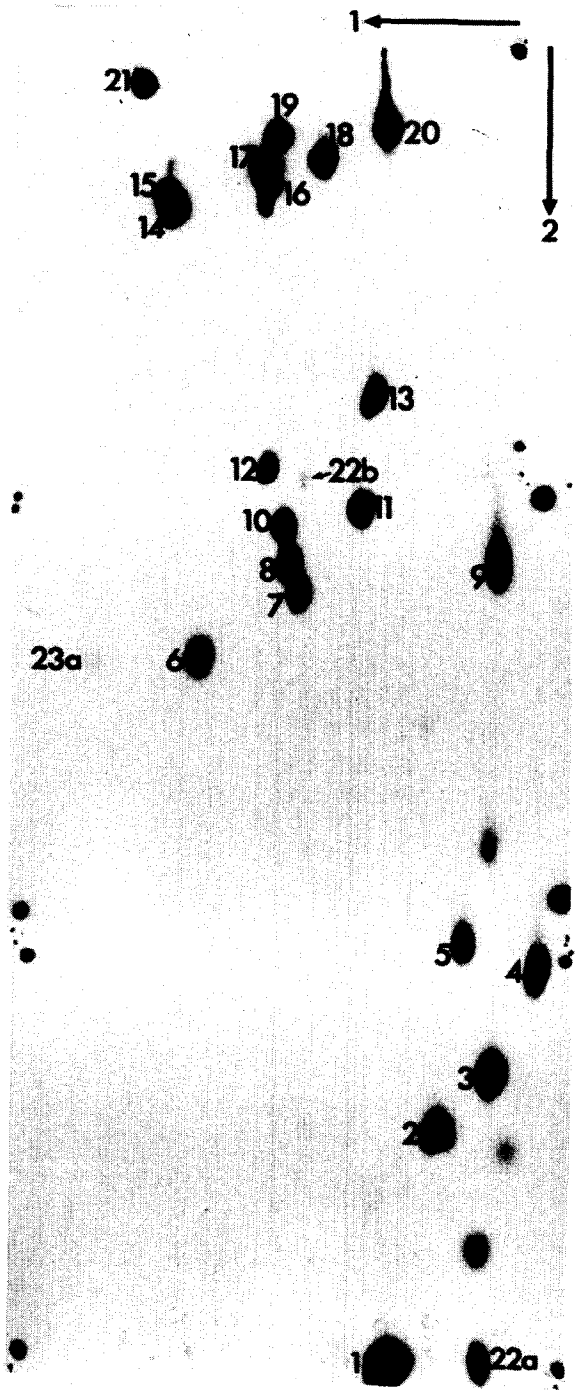

Fig.1. Autoradiograph of a two dimensional separation of the products of an RNAase T_1 digestion of turtle 5 S rRNA. Products were separated in the first dimension by electrophoresis on Cellogel in 7 M urea at pH 3.5. Electrophoresis in the second dimension was on DEAE-cellulose paper in 7% formic acid.

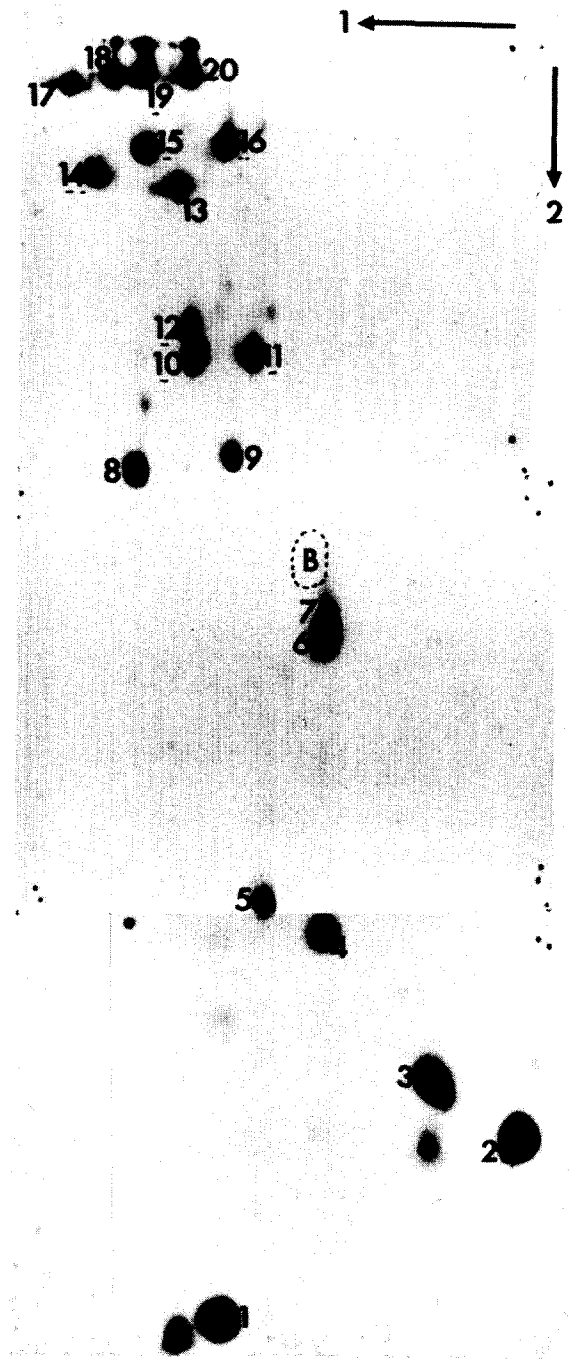

Fig.2. Autoradiograph of a two-dimensional separation of the products of a pancreatic RNAase digestion of turtle 5 S rRNA. The conditions used were as described in the caption to fig.1.

Table 1
Ribonuclease T_1 digestion products of turtle 5 S rRNA

Spot (fig.1)	Sequence	Methods used[a] (in addition to alakaline hydrolysis)	Molar yield[b]
t-1	Gp		15.6 (15)
t-2	A-Gp		1.3 (1)
t-3	C-A-Cp	PR → Cp + A-Gp	1.2 (1)
t-4	C-C-C-Gp		0.8 (1)
t-5	A-A-Gp		1.1 (1)
t-6	U-Gp		1.4 (1)
t-7	U-C-Gp	P-SVD → pC + pG	0.9 (1)
t-8	C-U-Gp	P-SVD → pU + pG	1.0 (1)
t-9	A-A-C-A-C-Gp[c]	PR → A-A-Cp + A-Cp + Gp, SPD → (2Ap,2Cp)Gp + (Ap,2Cp)Gp + (Ap,Cp)Gp	0.7 (1)
t-10	U-A-Gp	PR → Up + A-Gp	1.0 (1)
t-11	C-C-U-Gp	CMCT → U*-Gp + C-U*-Gp	1.1 (1)[d]
t-12	A-U-Gp	PR → A-Up + Gp	1.1 (1)
t-13	C-U-A-A-Gp	PR → Cp + Up + A-A-Gp, SPD → U-A-A-Gp	1.0 (1)
t-14	U-C-U-Gp	SPD → C-U-Gp + U-Gp	1.2 (1)
t-15	U-U-A-Gp	PR → 2Up + A-Gp	1.2 (1)
t-16	U-C-U-A-C-Gp	PR → 2Up + Cp + Gp + A-Cp, U_2 → (Cp,2Up)Ap + C-Gp, CMCT → U*-Cp + U*-A-Cp	0.9 (1)
t-17	A-U-C-U-C-Gp	PR → 2Cp + Up + Gp + A-Up, U_2 → (2Cp,2Up)Gp + Ap, CMCT → A-U*-Cp + U*-Cp	2.1 (2)
t-18	A-C-C-U-C-C-U-Gp[c]	PR → A-Cp + 3Cp + 2Up + Gp, U_2 → Ap + (4Cp,2Up)Gp + (Cp,Up)Gp (minor product)	1.1 (1)
t-19	A-A-U-A-C-U-Gp[c]	PR → A-A-Up + A-Cp + Up + Gp, U_2 → U-Ap + C-U-Gp	1.1 (1)
t-20	C-C-A-U-A-C-C-A-C-C-C-U-Gp	PR → 5-6Cp + Up + Gp + A-Up + 2A-Cp, U_2 → 2C-C-Ap + U-Ap + (3Cp,Up)Gp, CMCT → (2Ap,U*p)Cp + U*-Gp	0.7 (1)
t-21	U-A-C-U-U-Gp	PR → 3Up + Gp + A-Cp, U_2 → U-Ap + (Cp,2Up)Gp	1.0 (1)
t-22a	C-U-U	SVD → pU	0.7 } (1)
t-22b	C-U-U-U	SVD → pU	0.1 } (1)
t-23a	pGp		0.1
t-23b	ppGp		0.2 } (1)
t-23c	pppGp		0.6

[a] The abbreviations are: PR, pancreatic RNase digestion; SPD, spleen phosphodiesterase digestion; P-SVD, alkaline phosphatase treatment, followed by purification of the product and snake venom phosphodiesterase digestion; U_2, RNAase U_2 digestion; CMCT, modification of the oligonucleotide with *N*-cyclohexyl-*N'*-(β-morpholinyl-4-ethyl) carbodiimide methyl-*p*-toluene sulfonate followed by pancreatic RNase digestion. U* indicates a modified U

[b] The theoretical yield, based on the derived sequence, is given in parentheses. Experimental yields are based on averages of three experiments

[c] Not found in RNAase T_1 digests of mammalian 5 S rRNA

[d] Reduced from two moles in mammalian 5 S rRNA digests

Table 2
Pancreatic ribonuclease digestion products or turtle 5 S rRNA

Spot (fig.2)	Sequence	Methods used[a] (in addition to alkaline hydrolysis)	Molar yield[b]
p-1	Up		16.5 (15–16)
p-2	Cp		19.0 (17)
p-3	A-Cp		5.7 (6)[c]
p-4	G-Cp		2.2 (2)[d]
p-5	A-Up		1.5 (1)
p-6	G-A-A-Cp	$T_1 \rightarrow$ Gp + A-A-Cp	2.2 (1)
p-7	A-A-G-Cp	$T_1 \rightarrow$ A-A-Gp + Cp	2.2 (1)
p-8	G-Up		2.9 (2)
p-9	G-G-Cp		1.1 (1)
p-10	G-A-Up	$T_1 \rightarrow$ Gp + A-Up	2.1 (2)
p-11	A-G-G-Cp	$T_1 \rightarrow$ A-Gp + Gp + Cp, SPD → G-G-Cp + G-Cp	1.0 (1)
p-12	A-G-Up	$T_1 \rightarrow$ A-Gp + Up	1.2 (1)
p-13	G-G-G-Cp		0.6 (1)
p-14	G-G-Up		1.0 (1)
p-15	G-G-A-Up	$T_1 \rightarrow$ 2Gp + A-Up	0.9 (1)
p-16	G-G-A-A-G-Cp	$T_1 \rightarrow$ 2Gp + Cp + A-A-Gp, SPD → (2Gp,2Ap)Cp + (Gp, 2Ap)Cp + (Gp,Ap)Cp	1.2 (1)
p-17	G-G-G-Up		0.8 (1)
p-18	A-G-G-G-Up	$T_1 \rightarrow$ 2Gp + Up + A-Gp, SPD → G-G-G-Up + G-G-Up	0.8 (1)
p-19	G-G-G-A-A-Up	$T_1 \rightarrow$ 3Gp + A-A-Up	0.5 (1)
p-20	G-G-G-A-G-A-Cp	$T_1 \rightarrow$ 3Gp + A-Gp + A-Cp, SPD → (3Gp,2Ap)Cp + (2Gp,2Ap)Cp + (Gp,2Ap)Cp + (Gp,Ap)Cp	0.5 (1)
p-21a	pG-Up		0.1
p-21b	ppG-Up		0.2 (1)
p-21c	pppG-Up		0.6

[a]The abbreviations are: T_1, RNAase T_1 digestion; SPD, spleen phosphodiesterase digestion
[b]The theoretical yield, based on the derived sequence, is given in parentheses. Experimental yields are based on averages of three experiments
[c]Increased from five moles in pancreatic RNAase digests of mammalian 5 S rRNA
[d]Decreased from four moles in pancreatic RNAase digests of mammalian 5 S rRNA

products. The loss of G at position 93 explains the second missing GC in pancreatic RNAase digests.

The products of pancreatic RNAase and RNAase U_2 digestion of t-19 were sufficient to define its sequence and thus demonstrate the replacement of C by U at position 105.

4. Discussion

As shown in fig.3 the turtle 5 S rRNA sequence is very closely related to the mammalian and iguana sequences. The 5′-terminal region (bases 1–24) is identical to the mammalian sequence, in contrast to all other non-mammalian species so far examined. The presence of the A at position 25 is a feature which is found in the iguana sequence [11] as well as rainbow trout (Roy, unpublished data) and *Drosophila melanogaster* 5 S rRNA [15]. The transversion leading to a U at position 93 is a change which the turtle sequence has in common with the chicken [9,10]. In none of these studies has the relevant RNAase T_1 oligonucleotide been sequenced unambiguously, but the probability of any other sequence is very low. The alteration of the sequence at position 105 is unique to the turtle among the vertebrate species examined to date.

The only base change found in turtle 5 S rRNA which would affect the stability of a helical region in the model proposed by Fox and Woese [16] is

Sequence and position	Organism	Oligonucleotide
2 7		
U C U A C G	Human	
C C U A C G	Iguana	
U C U A C G	Turtle	t 16
22 27		
A A C G C G	Human	
A A C A C G	Iguana	
A A C A C G	Turtle	t 9
90 97		
A C C G C C U G	Human	
A C C G C C U G	Iguana	
A C C U C C U G	Turtle	t 18
100 106		
A A U A C C G	Human	
A A U A C C G	Iguana	
A A U A C U G	Turtle	t 19

Fig.3. – Comparisons of those regions of the turtle 5 S rRNA sequence which differ from the mammalian and/or iguana sequences.

the C → U transition at position 105. The sequence of more primitive eukaryotic species 5 S rRNA will have to be determined to test the generality of the Fox and Woese model as applied to eukaryotic 5 S rRNA's.

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